REMARKS

Claims 1 to 38 are pending in this application.

MEMORANDUM OF INTERVIEW

On February 12, 2007, the Examiner and Supervisory Examiner for this case, Ms. Shaw and Dr. Shukla, kindly agreed to and conducted a personal interview with the undersigned. The undersigned would like to thank the Examiners for the productive interview.

During the interview the obviousness rejection over Eads (NAR 2000) in view of U.S. Patent 6,258,568 to Nyren were discussed as well as draft claim amendments that had been faxed to the Office on February 10, 2007. These draft claims further highlighted that the real-time sequencing in (c) of , e.g., claim 1, was carried out using the previously amplified nucleic acid molecules. Applicants' representative pointed out that Eads' disclosure makes clear that the product of the RT-PCR in Eads' MethyLight method and the PCR product used for the Bisulfite Genomic Sequencing described on page (iii) differ. The undersigned in particular pointed out that the primers used for the PCR used for bisulfite genomic sequencing are situated "outside" the primers that are used to produced the final MethyLight product (Compare description on (ii), right column and (iii), left column).

Also discussed was the possibility of providing test results comparing (a) results obtained via real time (RT) sequencing Eads' real time (RT) PCR MethyLight products with (b) results obtained via the claimed invention.

Applicants were able to obtain RT-PCR products from the laboratory of Dr. Laird's (co-author of the Eads' reference and head of the laboratory in which the experiments leading to the Eads paper were performed), the only laboratory the inventors and Dr. Laird are aware of having firmly established the MethyLight method. Unfortunately, the comparative experiments could not be timely performed for health reasons.

However, theoretical considerations in anticipation of the experiments resulted in a reformulation of the claims that more clearly point out the differences between (i) a combination of Eads and Nyren and (ii) the present invention. These reformulated claims are discussed and presented herein.

Also discussed during the interview was the rejection of claim 34. As best understood by the undersigned, the Examiners noted that the reference to "inherency"

on page 14 of the Action was made to support the Office's 35 USC §112 rejection, rather than a rejection as discussed in MPEP §2112, IV.

35 U.S.C. §112 Rejections

In paragraph 2, the Office rejected claim 34 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Office rejected the phrase "*wherein an allele frequency of 5% can be detected*" as not being an active process step and not further limiting the method of claim 10. The Office expressed the opinion that the phrase appears to be a property of the method of claim 10.

The Office also stated that the application did not provide any evidence that the method can actually detect an allele frequency of 5%.

Applicants would first like to direct the Office attention to applicants' remarks on page 10 of applicants' July 14, 2006 response and to paragraph 1 on page 24 of the application referred to therein for support of this claim limitation. Here the specification states, referring to the Figure 2:

"A minor allele frequency of 5% could be detected without any problem. The SD obtained with SNaPmeth ranged between 0% and 3.7%, in the PyroMeth assay between 0.2% and 1%."

See also, In re Wertheim, 541 F.2d 257, 263,191 USPQ 90, 97 (CCPA 1976).

Accordingly, applicants have provided the required evidence to support this limitation.

Furthermore, in response to the Office's indefiniteness rejection, applicants have now amended claim 34 to provide an "active process step" namely a detection "step." (MPEP §2173.05(a)).

Applicants also note that in §2173.02, the MPEP points out that the focus in an 35 USC §112, second paragraph definiteness analysis should be on whether the claim meets the threshold requirements of clarity and precision. The MPEP notes that claims which define the patentable subject matter with a reasonable degree of particularity and distinctness should be allowed. Otherwise, Examiners are encouraged to suggest claim language to applicants to improve the clarity or precision of the language used (MPEP §2173.02).

In *Marley Mouldings v. Mikron Industries,* 417 F.3d 1356 (Fed. Cir. 2005), the Federal Circuit, citing *Bancorp Servs., LLC*, noted that when a claim "is not insolubly ambiguous, it is not invalid for indefiniteness."

Applicants respectfully submit that claim 34 as amended clearly meets the threshold requirement of clarity and precision.

35 U.S.C. §103 Rejection

In paragraph 4, the Office continued to reject claims 1 to 12 and 19 to 28 and 30 to 34 under 35 USC §103(a) as being obvious over Eads et al., NAR 28(8):e32 (i) to (viii) (2000) (hereinafter "Eads") in view of U.S. Patent 6,258,568 to Nyren et al. (hereinafter "Nyren").

Applicants have further amended the claims to highlight the differences between a combination of Eads and Nyren and the present invention.

Claim 1 now requires:

> (b) amplifying said nucleic acid molecule treated with said agent <u>via at least one amplification primer,</u>
>
> <u>wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of single stranded amplified nucleic acid molecules</u> to generate <u>a single stranded</u> amplified nucleic acid molecule;
>
> (c) real-time sequencing **<u>said single stranded amplified nucleic acid molecule</u>**" *[emphasis added]*

The newly added language emphasized, among others, that the nucleic acid amplified in (b) is being sequenced in (c). Claims 12 and 32 were similarly amended.

Support for this claim amendment can be found on page 13, first full paragraph ([0032] of publication).

In contrast, Eads does not disclose that "**<u>said</u>** . . . amplified nucleic acid molecule" is sequenced. Rather a separate PCR product is sequenced to analyze the MLH1 MethyLight amplicon. In fact, prior to sequencing, the PCR products of Eads are cloned and the individual clones are sequenced. As can be seen from Fig. 6 and the paragraph on page iii describing the genomic bisulfite sequencing method, Eads generates a new PCR product using different primers, in particular primers that are

situated outside the MLH1 amplicon. (compare "genomic bisulfite sequencing" primers disclosed on page iii to "MethyLight" primers disclosed on page ii).

The above amendment to the claims also highlights that the amplification primer is detectably labeled with a detectable label that forms an anchor for removal of single stranded amplified nucleic acid molecules. The single stranded amplified nucleic acid molecule obtained is then real-time sequenced. Eads uses real-time (RT) PCR (TaqMan®) for her amplification step. Applicants are not aware of and could, upon some investigation, not find any work in which so labeled primers could be successfully used in RT- PCR (TaqMan®)). In fact, applicants found a German dissertation in which use of biotin labeled primers for RT-PCR are discussed. The author states on page 87 (German original attached):

> "Experiments demonstrated that, in the real-time PCR, disturbances due to enhancer or quenching effects can result from the biotin-molecules of the primer or of individual polystyrol pellets (data not shown). To avoid this, biotin containing primers should, if possible, be removed from the system."

Excerpt from: *"Qualitative and quantitative detection of nucleic acids from entric viruses in the environment as an instrument for risk value*," Daniela Pusch, Freie Universität Berlin, Germany, *Digital Dissertation*, http://www.diss.fu-berlin.de/2006/49/index.html (26.02.2006).

Applicants have shown above that nothing in Eads and Nyren suggests to the person skilled in the art, employing his/her skill and common sense, a combination of all of the elements of independent claims 1, 12 and 32. In fact several factors speak against such a combination:

(1) The shortage of literature discussing successful performance of Eads' RT-PCR with detectably labeled primers suggests that Eads cannot be upgraded to the invention as presently claimed. Literature as recent as 2006 discourages employing detectably labeled (i.e., biotin) primers for the RT-PCR that Eads' uses for amplifying her nucleic acids (*KSR v. Teleflex, 04-1350* (U.S. Supreme Court, April 30, 2007, page 22, paragraph 2));

(2) Eads' MethyLight method is completed with the performance of the RT-PCR (see Abstract: "This study describes a *high-throughput quantitative methylation assay*

that utilizes fluorescence-based real-time PCR technology (TaqMan®) that requires *no further manipulations after the PCR step*" *[emphasis added]*). The genomic bisulfite sequencing method was performed by Eads to confirm the results obtained with the MethyLight method. Eads cloned the MethyLight amplicon and performed a separate PCR of the entire amplicon which was then sequenced. He makes no mentioning that there might be any advantage in sequencing the RT-PCT product of the MethyLight method itself, certainly not in real-time sequencing the RT-PCT product of the MethyLight method.

Claim 10:

In response to the Office's rejection of claim 10 on page 7 of the Office Action, applicants have amended claim 10. The amendment is supported by paragraph bridging pages 8 and 9 of the specification.

The claim makes now clear that the calculation is based on results from real time sequencing and not a real time PCR as discussed in Eads and relied on in the Office Action. Claim 25 was amended accordingly.

Claim 12:

In response to the Office's rejection of claim 12 on page 7 of the Office Action, applicants have amended claim 12 to more clearly conform the body of the claim with the preamble. The preamble also provides the basis for this amendment of paragraph (d) of the claim.

Applicants submit that the amendment should fully address the Offices concern and allow full consideration of this claim limitation.

Claim 32:

Similarly, claim 32 now clearly recites in (c) that new nucleotide pairing partners are generated. Accordingly, the language of the preamble has been introduced into the body of the claim, clearly limiting the same. Thus, the claim has been further amended to more clearly define the invention. The Office is directed to page 8, second full paragraph of the application as filed for support. The remaining amendments to claim 32 have been discussed and support in the application has been provided in the context of the discussion of the obviousness rejection above.

Claim 34:

This claim, in particular the rejection under 35 USC §112 and where support can be found, has been discussed on pages 11 and 12 of this response.

During the interview the Office noted that the reference to inherency on page 14 of the Office Action was not aimed at inherency as set forth in MPEP §2112, IV. However, in case that the inherency rejection should nonetheless be maintained, applicants note that the Examiner has not provided a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art as required by MPEP §2112, IV.

The Office expressed in this context the opinion that Nyren et al teach that their invention can be used both to identify and quantitative selectively amplified DNA fragments as well as be used for detection of single base substitutions and for estimation of the heterozygosity index for an amplified polymorphic gene fragment. Thus, the conclusion was drawn that the method can be used to screen for rare point mutations (paragraph bridging page 19 to 20 of the Office Action).

Applicant would like to direct the Office to applicants' response of July 14, 2006, were it was noted on page 14:

"In fact Nyren notes at a number of places that his method is prone to substantial background noises (column 7, lines 48 to 55: since nucleotide dATP is a substrate for apyrase; column 10, lines 5 to 6: in view of the digestion of 3' ends by proof-reading-polymerases; column 10, lines 16 to 19: accumulation of final products during sequencing cycles; column 12, lines 38 to 41: due to the contamination of, for example, dNTP-charged with PP_i, see also Figs. 3 and 4). Applicants submit that such a propensity to background noise renders it unlikely that with Nyren's method the sensitivities of the invention expressed by claim 34 could be accomplished. Background is particularly disturbing if the base distribution of the sequence subject to sequencing does not correspond to a 50/50 distribution, which is common in DNA methylation. In this context, applicants note that while Nyren mentions quantitative detection of signals (column 10, lines 49 to 62), he refers here only to a discrimination at an allele distribution of 50% (column 10, lines 49 to 53)."

Thus, applicants submit that the disclosure of Nyren does not provide adequate support for the Office's rationale.

New Claims 35 and 36:

New claim 35 states that the primer:

"does not comprise CpG dinucleotides"

This claim limitation is supported, e.g., on page 21, paragraph 1.3 entitled "PCR." ([0071] of publication)

Eads primers and probes, which are used to in fact determine the methylation status of an nucleic acid molecule, are specifically designed to overlap potential CpG dinucleotide sites (one to five potential CpGs) as described under the heading "MethyLight primer and probe sequences" on page ii of Eads. (Compare control on page iv, A: These primers and probes are not used to determine the methylation status of the respective nucleic acid molecule).

The absence of CpG dinucleotides in the primers of the invention as claimed in claim 35 renders them to be non-discriminatory as to the template's CpG methylation status. As a results and as opposed to, e.g., Eads, this embodiment allows accurate calculation/quantification of the original CpG methylation status since the amplification product is not biased. In Eads such a bias would occur since the experimentator choosing Eads CpG containing primers would have to assume that the respective CpGs are either methylated or not.

As highlighted in new claim 36, this experimental set up also allows that all nucleotides formerly methylated or not methylated in the nucleic acid molecules are detected. New claim 36 states:

> "all nucleotides formerly methylated or not methylated in said nucleic acid molecule are detected."

New claim 36 is supported by the paragraph bridging pages 8 and 9 of the disclosure ([0022] of publication).

In paragraph 5, claims 13 to 16, 18 and 29 are continued to be rejected under 35 USC §103(a) as being obvious over Eads in view of Nyren and further in view of United States Patent No. 5,786,146 to Herman (hereinafter "Herman").

In paragraph 6, claim 17 remained rejected as being obvious over Eads in view of Nyren and further in view of United States Patent Publication No. 2003/0232351 to Feinberg (hereinafter "Feinberg").

The deficiencies of Eads and Nyren have been discussed above. Neither Herman nor Feinberg cure these deficiencies.

Also, applicants have added new claim 38 which refers to a neurodegenerative disease or another neurological disorder.

Applicants have shown above that independent claims 1, 12 and 32 are non-obvious in view of Eads when combined with Nyren. These claims should therefore be in condition for allowance. Claims 2 to 5, 7 to 11, 13 to 20, 22 to 31 and 33 to 38 that are dependent therefrom should also be in condition for allowance.

The undersigned sincerely urges the Office to call her at the number provided below to discuss any issues that might arise in the further prosecution of this case.

The fee for additional two claims in excess of 20 is submitted herewith. However, the Commissioner is authorized to charge or credit deposit account no. 50-3135 as required.

Respectfully submitted,

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